

CLAIMS

1. A method for synthesizing non-photosynthetic carotenoids chosen from β -carotene, canthaxanthin or astaxanthin, using photosynthetic bacteria which produce at least one photosynthetic carotenoid, one of the synthesis intermediates of which is lycopene, characterized in that it comprises the following steps:
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- 10 i. deleting, in the bacteria, at least one part of one or more genes involved in the endogenous synthesis pathway which follows that of lycopene, so as to stop said synthesis at the level of lycopene,
- 15 ii. inserting the following genes:
- either crtY if the carotenoid to be synthesized is β -carotene,
 - or crtY and crtW if the carotenoid to be synthesized is canthaxanthin or β -carotene,
 - 20 - or crtY, crtZ and crtW if the carotenoid to be synthesized is astaxanthin or β -carotene,
- iii. culturing said bacteria thus modified, and
- 25 iv. extracting the carotenoid(s) contained in the bacteria.
2. The method as claimed in claim 1, for synthesizing canthaxanthin or astaxanthin, characterized in
- 30 that the culturing conditions are sequential and comprise the following steps:
- c. culturing said bacteria thus modified firstly under anaerobic conditions under light,
 - d. then, secondly, under aerobic conditions, in
 - 35 the dark.

3. The method as claimed in claim 1, for synthesizing β -carotene, characterized in that the culturing conditions are as follows:
 - b. culturing said bacteria thus modified, under
5 anaerobic conditions under light.
4. The method as claimed in claim 2, characterized in that steps a and/or b are carried out under microaerobic conditions under light.
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5. The method as claimed in claim 4, characterized in that, under microaerobic conditions, the dioxygen percentage is between 1% and 10%, preferably 3% to 8%, limits included.
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6. The method as claimed in any one of claims 2 to 5, characterized in that steps a and b are successively repeated.
- 20 7. A method for synthesizing non-photosynthetic carotenoids chosen from β -carotene, canthaxanthin or astaxanthin, using photosynthetic bacteria which produce at least one photosynthetic carotenoid, one of the synthesis intermediates of which is lycopene, characterized in that the
25 method comprises the following steps:
 - i. using mutants of photosynthetic bacteria in which the photosynthesis is no longer repressed by dioxygen,
 - 30 ii. deleting, in the bacteria, at least one part of one or more genes involved in the endogenous synthesis pathway which follows that of lycopene, so as to stop said synthesis at the level of lycopene,
 - 35 iii. inserting the following genes:
 - either crtY if the carotenoid to be synthesized is β -carotene,

- or crtY and crtW if the carotenoid to be synthesized is canthaxanthin or β -carotene,
 - or crtY, crtZ and crtW if the carotenoid to be synthesized is astaxanthin or β -carotene,
- iv. culturing said bacteria thus modified, under aerobic or microaerobic conditions, in order to synthesize canthaxanthin or astaxanthin, or culturing under anaerobic conditions in order to synthesize β -carotene, and
- v. extracting the carotenoid(s) contained in the bacteria.
8. The method as claimed in claim 7, characterized in that the mutants in which the photosynthesis is no longer repressed by dioxygen are obtained by deletion of the gene encoding the PpsR transcription factor.
9. The method as claimed in any one of the preceding claims, characterized in that the bacteria are of the *Rhodopseudomonas* genus, preferably of the species *Rhodopseudomonas palustris*.
10. The method as claimed in any one of claims 1 to 8, characterized in that said photosynthetic bacteria which produce at least one photosynthetic carotenoid, one of the synthesis intermediates of which is lycopene, are obtained from photosynthetic bacteria which produce at least one photosynthetic carotenoid, one of the synthesis intermediates of which is phytoene, phytofluene, ζ -carotene or neurosporene, said bacteria having optionally undergone a deletion or disruption of the endogenous crtI gene, followed by insertion of an exogenous crtI encoding a phytoene desaturase ensuring 4 successive phytoene desaturation steps.

11. The method as claimed in any one of the preceding claims, characterized in that the insertion of the crtY, crtZ and/or crtW genes is carried out in the zone of the genes at least partially deleted.
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12. A photosynthetic bacterium which produces, in an alternating or concomitant manner, at least lycopene, β -carotene and canthaxanthin or astaxanthin, characterized in that said bacterium
- 10 can be obtained by means of the method as claimed in claim 1 or claim 9 in terms of its relation to claim 1.
13. A photosynthetic bacterium, characterized in that
- 15 it is obtained according to the following method:
- i. culturing a photosynthetic bacterium which synthesizes at least one photosynthetic carotenoid, the synthesis intermediate of which is lycopene,
 - 20 ii. deleting at least a part of one or more genes involved in the endogenous synthesis pathway which follows that of lycopene, so as to stop said synthesis at the level of lycopene,
 - iii. inserting the following genes:
- 25 - either crtY if the carotenoid to be synthesized is β -carotene,
 - or crtY and crtW if the carotenoid to be synthesized is canthaxanthin or β -carotene,
 - 30 - or crtY, crtZ and crtW if the carotenoid to be synthesized is astaxanthin or β -carotene.
14. A mutant of a photosynthetic bacterium,
- 35 characterized in that it is obtained according to the following method:
- i. using a mutant of a photosynthetic bacteria synthesizing at least one photosynthetic carotenoid, the synthesis intermediate of

- which is lycopene, in which the photosynthesis is no longer repressed by dioxygen, producing canthaxanthin or astaxanthin,
- 5 ii. deleting at least a part of one or more genes involved in the endogenous synthesis pathway which follows that of lycopene, so as to stop said synthesis at the level of lycopene,
- iii. inserting the following genes:
- 10 - either crtY if the carotenoid to be synthesized is β -carotene,
- or crtY and crtW if the carotenoid to be synthesized is canthaxanthin or β -carotene,
- 15 - or crtY, crtZ and crtW if the carotenoid to be synthesized is astaxanthin or β -carotene.
15. The bacterium as claimed in claim 12 or 13, or the
- 20 mutant as claimed in claim 14, characterized in that it is respectively obtained from a bacterium or from a mutant which produces at least one photosynthetic carotenoid, one of the synthesis intermediates of which is phytoene, phytofluene,
- 25 ζ -carotene or neurosporene, said bacterium or mutant having optionally undergone a deletion or disruption of the endogenous crtI gene, followed by insertion of an exogenous crtI gene encoding a phytoene desaturase ensuring 4 successive phytoene
- 30 desaturation steps.